Human Reproduction Vol.20, No.1 pp. 170–174, 2005
Advance Access publication November 26, 2004
doi:10.1093/humrep/deh573

Minimizing embryo expulsion after embryo transfer: a randomized controlled study

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BACKGROUND: The aim of this work was to modify the embryo transfer technique to prevent expulsion of the embryos by exerting gentle mechanical pressure on the cervix using the vaginal speculum. METHODS: A total of 639 infertile patients undergoing ICSI were prospectively randomized into two groups using sealed dark envelopes. In the study group (*n* = 325) the screw of the vaginal speculum was loosened in order to exert a gentle pressure on the portiovaginalis of the cervix before ejecting the embryos, and was maintained for 7 min afterwards. In the control group (*n* = 314) no pressure was applied on the cervix during embryo transfer and the vaginal speculum was removed after transferring the embryos. RESULTS: The clinical pregnancy rate was significantly higher in the study group than in the control group [207/325 (67%) versus 150/314 (47.8%); odds ratio (OR) 1.39; 95% confidence interval (CI) 1.11–1.74]. The implantation rate was also significantly higher in the study group [304/913 (33.3%) versus 198/920 (21.5%); OR 1.54; 95% CI 1.26–1.89]. CONCLUSIONS: Applying gentle mechanical pressure on the portiovaginalis of the cervix using the vaginal speculum during and after transferring the embryos significantly improved clinical pregnancy and implantation rates.

Key words: embryo transfer technique/ICSI/implantation rate/IVF/pregnancy rate

Introduction

Transferring the embryos into the uterine cavity is the last and most critical step in IVF. It is routinely done through the transcervical route and is associated with multiple potential negative factors (Mansour and Aboulghar, 2002). One of these negative factors is the initiation of uterine contractions at the time of embryo transfer. Passing the catheter through the internal cervical os may initiate reflex uterine contractions (Fanchin *et al.*, 1998), possibly through the release of prostaglandins (Fraser, 1992). It has been demonstrated that ~15% of the transferred embryos are expelled after embryo transfer, and could be collected from the tip of the catheter, external cervical os and the vaginal speculum (Poindexter *et al.*, 1986).

The aim of this work is to modify the embryo transfer technique to prevent expulsion of the embryos from the uterine cavity after embryo transfer by applying gentle mechanical pressure on the portiovaginalis of the cervix using the vaginal speculum.

Materials and methods

Participants

Patients included in the study were infertile couples undergoing their first ICSI trial for male factor infertility at the Egyptian IVF-ET Center. The female partners were <39 years old with a normal hormonal profile and no pelvic pathology. Azoospermic patients requiring surgical retrieval of spermatozoa were excluded from the study. Ethical approval for the study was obtained from the institutional review board. The period of study was from January 2001 to January 2002.

Design and procedures

The pituitary was down-regulated using long-protocol GnRH agonist analogue. It was given in the form of subcutaneous tripterolin 0.1 mg/day (decapeptyl; Ferring, GmbH, Kiel, Germany) starting in the late luteal phase until HCG injection. After pituitary down-regulation was confirmed by estradiol level <50 pg/ml, ovarian stimulation was started by giving HMG 150–300 IU/day (Menogone; Ferring) for 7 days, and then the dose was modified according to the response. HCG (10 000 IU) (Pregnyl; Nile Co., Cairo, Egypt) was given intramuscularly when the leading three follicles reached 18 mm in mean diameter. Oocyte pick-up was carried out 36 h after HCG injection through the ultrasonic transvaginal route. Oocyte injection and embryo culture was as described previously (Mansour *et al.*, 1996).

Embryo transfer was performed 48–72 h after oocyte pick-up by the same gynecologist (R.M.) for this study. Eligible patients were randomly allocated to the study or control group using sealed dark envelopes prepared by the embryologist. Details of the recruitment and randomization of patients are given in the flow diagram (Figure 1). In both groups, the embryo transfer was done at the lithotomy position. The previously taken ultrasound picture of the uterus and dummy embryo transfer (Mansour *et al.*, 1990) were revised to get an idea of the length and direction of the uterine cavity. After visualizing the portiovaginalis of the cervix using Cusco's speculum,

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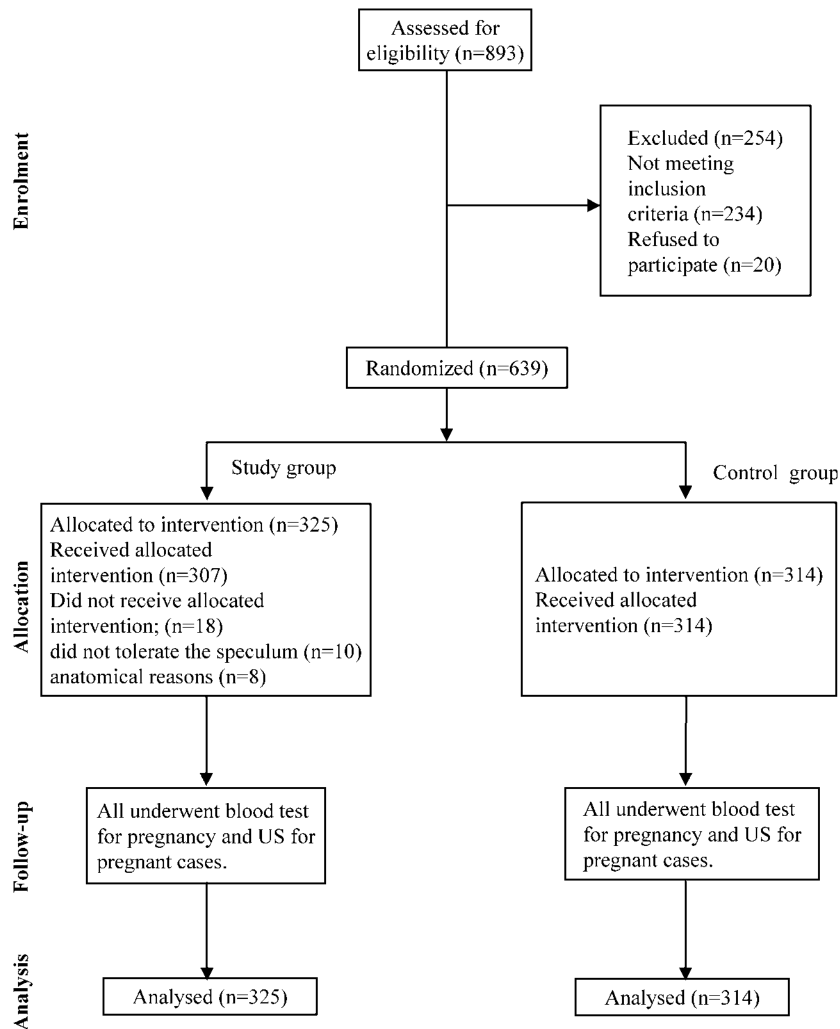

Figure 1. The number of patients eligible for the study, randomized, study group and control group, received the allocated treatment.

the cervix was cleaned using sterile gauze and tissue culture media (DPBS or Ham's F10). The cervical mucus was aspirated gently and repeatedly at the external cervical os using a 1 ml syringe. A dummy embryo transfer was done using a sterile Wallace catheter (1816 N, HG; Wallace Ltd, Colchester, UK) to make sure that it could be introduced easily through the internal os. If it could not be introduced, a more rigid catheter (Cook, Queensland, Australia) was tried. After performing the dummy embryo transfer, the embryos were loaded into a new catheter, either Wallace or Cook according to the dummy embryo transfer, as follows. The embryo transfer catheter was rinsed then filled with tissue culture medium. About 10–20 ml tissue culture medium was aspirated, then the embryos (up to three) were aspirated in ~10–20 ml tissue culture medium so that the embryos would be away from the tip of the catheter. If the Wallace catheter was used, the soft internal catheter, protruding from the external rigid sheath, was introduced gently through the internal cervical os and stopped ~1 cm below the fundus. The outer rigid sheath was stopped just at the internal cervical os and not pushed beyond it. If the Cook catheter was used, the tip of the inner catheter was positioned flush with the external sheath until it passed the internal cervical os, then the internal sheath one was advanced 2 cm into the uterine cavity. The uterine cavity length and direction was determined previously by ultrasonography and a dummy embryo transfer.

In the study group, after introducing the embryo transfer catheter, the screw of the vaginal speculum was loosened so that its two lips would close gently on the portiovaginalis of the cervix (Figure 2). At this point, after passing the internal os and loosening the speculum to close on the cervix, some patients experienced suprapubic heaviness, mild discomfort or cramping. After waiting for ~1–2 min, when this complaint disappeared, the embryos were

Figure 2. Study group. The two valves of the vaginal speculum closed on the cervix.

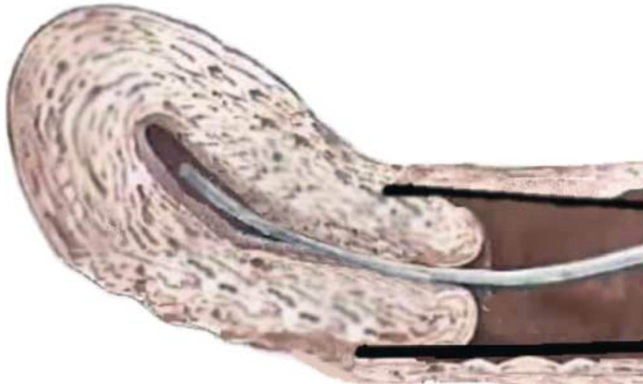

Figure 3. Control group. The two valves of the vaginal speculum are open.

ejected into the uterine cavity, the plunger of the syringe was kept pressed, and the embryo transfer catheter was withdrawn very slowly in gradual spiral movements. Then, the vaginal speculum was left in place pressing on the cervix after withdrawal of the catheter for 5–7 min before removal.

In the control group, the procedure was performed as in the study group, except that after introducing the embryo transfer catheter through the internal os and stopping 1 cm short of the fundus, the embryos were ejected into the uterine cavity and the catheter was withdrawn very slowly. In contrast to the study group, the screw of the vaginal speculum was not loosened to close on the portiovaginalis (Figure 3), and it was removed after withdrawal of the catheter. After completion of the embryo transfer in both groups, the patients stayed in bed for ~4–6 h before going home. Luteal phase support was given in the form of progesterone (Steris, Phoenix, AZ, USA) 100 mg as an intramuscular injection daily. Serum β-HCG test was done 2 weeks after embryo transfer and ultrasound was done 3 weeks later after a positive pregnancy test.

Statistical analysis

Data were collected on standard sheets and entered into a database.

Data are presented as mean ± standard deviation. Different outcome measures were compared using Student's *t*-test or χ^2-test where appropriate. *P*-values <0.05 were considered to be significant. Statistics were done using Arcus Quickstat (version I).

The sample size of 654 women provides 80% power and a two-sided significance level of 0.05 to test whether the modified embryo transfer technique is equivalent or even superior to the control group in an IVF/embryo transfer program. This sample size has adequate power to detect a difference of 10% in pregnancy rate/treated cycle.

Results

The study included 639 patients who underwent embryo transfer procedures by the same gynecologist (R.M.) in the period between from January 2001 to January 2002. There was no significant difference in the mean age, infertility period, number of oocytes retrieved, metaphase II oocytes and oocytes fertilized in both groups (Table I). There was no significant difference in the number of HMG ampules used in both groups. There was no significant difference in the ratio of catheter type per total number in each group [Cook catheter was used in 36/325 (11%) in study group versus 32/314 (10%) in the control group]. A total of 357 clinical pregnancies resulted, achieving a pregnancy rate per embryo transfer of 55.9%. The clinical pregnancy rate was significantly higher in the study group as compared with the control group [207/325 (67%) versus 150/314 (47.8%); odds ratio (OR) 1.39; 95% confidence interval (CI) 1.11–1.74]. The implantation rate was also significantly higher in the study group [304/913 (33.3%) versus 198/920 (21.5%); OR 1.54; 95% CI 1.26–1.89] (Table II). It was tolerable for all patients in the study group to have the speculum left in place for an average of 7 min after transferring the embryos, except for eight patients who felt uncomfortable, and the speculum had to be removed. In 10 more cases it was not possible to hold the portiovaginalis inbetween the two valves of the speculum for anatomical reasons. The mean time that the speculum was left in place after transferring the embryos in the study group

Table I. Patient's characteristics, ovarian response and fertilization

	Study group	Control group	*P*-value
Number of ET procedures	325	314	
Age (years)			0.13 (NS)
Mean	31.22 ± 4.66	31.59 ± 4.34	
Range	18–39	21–39	
Duration of infertility (years)			0.49 (NS)
Mean	6.75 ± 4.41	6.73 ± 4.36	
Range	1–21	1–19	
Number of HMG ampoules			0.98 (NS)
Mean ± SD	37.08 ± 13.19	37.04 ± 13.51	OR 1.01
Range	10–96	14–90	95% CI 0.62–1.62
Number of oocytes retrieved	4009	4026	0.36 (NS)
Mean ± SD	12.7 ± 6.9	12.7 ± 6.9	
Range	5–39	4–36	
MII oocytes	3158	3071	0.34 (NS)
Mean ± SD	9.99 ± 5.37	9.67 ± 5.37	OR 1.03
Range	4–28	3–25	95% CI 0.96–1.10
2PN oocytes	2094	1970	0.27 (NS)
Mean	6.69 ± 3.74	6.31 ± 4.03	OR 1.07
Range	3–20	3–24	95% CI 0.98–1.15
Fertilization rate (%)	66.3	64.2	0.08 (NS)

ET = embryo transfer; NS = not significant; SD = standard deviation; MII = metaphase II; 2PN = two pronuclei.

Table II. Outcome of treatment

	Study group	Control group	*P*-value
No. transferred embryos	913	920	(NS) 0.73
Mean ± SD per patient	2.91 ± 0.6	2.89 ± 0.69	OR 0.95 95% CI 0.83–1.04
No good quality embryos (grade I, II)	821	829	(NS) 0.87
Mean ± SD per patient (%)	2.16 ± 0.87 (89.92)	2.30 ± 0.87 (90.11)	OR 0.99 95% CI 0.87–1.14
No. poor quality embryos, (grade III)	92	91	(NS) 0.44
Mean ± SD per patient (%)	1.83 ± 1.00 (10.08)	1.87 ± 0.97 (9.89)	OR 1.02 95% CI 0.75–1.38
Implantation rate (%)	304/913 (33.3)	198/920 (21.5)	0.00002 OR 1.54 95% CI 1.26–1.89
Clinical pregnancy rate per transfer (%)	207/325 (67.4)	150/314 (47.8)	0.009 OR 1.39 95% CI 1.11–1.74
Multiple pregnancy rate (%)	79/207 (38.2)	38/150 (25.3)	0.09 (NS) OR 1.35 95% CI 0.88–2.07

NS = not significant; SD = standard deviation.

was 6.51 ± 2.92 min. The time taken to perform the whole embryo transfer procedure including dummy embryo transfer, loading the catheter and actual embryo transfer was 28.33 ± 6.37 min in the study group and 20.42 ± 3.45 min in the control group.

Discussion

Immediate or delayed expulsion of embryos after transferring them into the uterine cavity has always been a major concern in assisted reproduction (Harper *et al.*, 1961; Menezo *et al.*, 1985; Poindexter *et al.*, 1986; Schulman, 1986; Meldrum *et al.*, 1987). In two different studies mimicking embryo transfer, one using a radio opaque dye (Knutzen *et al.*, 1992) and the other using a methylene blue dye (Mansour *et al.*, 1994), it was found that the dye was extruded from the uterine cavity in 42% of the cases. Using artificial dried embryos for training in IVF, it was also found that only 45% of the embryos were present within the uterine cavity 1 h after the transfer (Menezo *et al.*, 1985). It has also been observed that after embryo transfer, the embryos can as easily move towards the cervical canal as towards the Fallopian tubes (Woolcott and Stanger, 1997; 1998). Moreover, ~15% of the embryos were found to be extruded after embryo transfer (Poindexter *et al.*, 1986). The presence of endometrial movements has been recognized by several groups (Birnholz, 1984; Ijland *et al.*, 1996). Stimulation of the cervix can lead to the release of oxytocin and consequently induces uterine contractility. In a prospective clinical study by Dorn *et al.*, (1999), serial blood samples were collected in time intervals of 20 s during the embryo transfer procedure to measure oxytocin concentration in serum. It was found that when a tenaculum was used, oxytocin was temporarily elevated until the end of the embryo transfer procedure. When a tenaculum was not used, no increase in serum oxytocin concentration was observed. These findings from previous studies have raised concern about the possibility of losing embryos after embryo transfer, and the motive behind this study was to try to develop a technique to reduce embryo expulsion after embryo transfer.

The idea behind this study is very simple. It depends on applying gentle pressure on the cervical canal at the time of the reflex uterine contractions that result from passing the embryo transfer catheter through the internal os, because these contractions may lead to the extrusion of the embryos after embryo transfer. By closing the vaginal speculum, light pressure from the two valves of the speculum is applied on the portiovaginalis of the cervix. This results in gently pressing the anterior and posterior lips of the cervix together, possibly obstructing the cervical canal up to the internal os. This simple technique significantly improved the pregnancy rate when compared with the control group.

The study does not suggest a mechanism by which the higher pregnancy rate was obtained in the study group. Only a methodological approach looking at the outcome of mock embryo transfer demonstrating displacement of markers following occlusion or no occlusion of the cervix might allow an explanation. The improvement in the results may have resulted from phenomena not directly linked to attempting to occlude the cervix, such as the increase in wait time before ejecting the embryos.

It was reasonably tolerable for the patients to have the speculum left in place for an average of 6.5 min after embryo transfer. The time taken to perform embryo transfer in the study group was only an average of 8.5 min longer than in the control group, and did not make a difference to the patient or the doctor.

In conclusion, this is a simple modification of the embryo transfer technique that significantly improved the implantation and clinical pregnancy rates. Since the completion of the study, this modified embryo transfer technique has been a routine procedure for all cases. The method is to apply gentle mechanical pressure on the portiovaginalis of the cervix using the vaginal speculum before and after ejecting the embryos into the uterine cavity to prevent their extrusion. Further experimental studies are being undertaken using

dummy embryo transfer to explain the mechanism of action of this technique.

References

Birnholz JC (1984) Ultrasonic visualization of endometrial movements. Fertil Steril 41,157–158.

Dorn C, Reinsberg J, Schlebusch H, Prietl G, van der Ven H and Krebs D (1999) Serum oxytocin concentration during embryo transfer procedure. Eur J Obstet Gynecol Reprod Biol 87,77–80.

Fanchin R, Righini C, Olivennes F, Taylor S, de Ziegler D and Frydman R (1998) Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. Hum Reprod 13,1968–1974.

Fraser IS (1992) Prostaglandins, prostaglandin inhibitors and their roles in gynaecological disorders. Baillieres Clin Obstet Gynaecol 6,829–857.

Harper MJK, Bennett JP and Rowson LE (1961) A possible explanation for the failure of non-surgical ovum transfer in the cow. Nature 190,789–790.

Ijland MM, Evers JL, Dunselman GA, van Katwijk C, Lo CR and Hoogland HJ (1996) Endometrial wavelike movements during the menstrual cycle. Fertil Steril 65,746–749.

Knutzen V, Stratton CJ, Sher G, McNamee PI, Huang TT and Soto-Albors C (1992) Mock embryo transfer in early luteal phase, the cycle before in vitro fertilization and embryo transfer: a descriptive study. Fertil Steril 57,156–162.

Mansour RT and Aboulghar MA (2002) Optimizing the embryo transfer technique. Hum Reprod 17,1149–1153.

Mansour R, Aboulghar M and Serour G (1990) Dummy embryo transfer: a technique that minimizes the problems of embryo transfer and improves the pregnancy rate in human in-vitro fertilization. Fertil Steril 54,678–681.

Mansour RT, Aboulghar MA, Serour GI and Amin YM (1994) Dummy embryo transfer using methylene blue dye. Hum Reprod 9,1257–1259.

Mansour RT, Aboulghar MA, Serour GI, Fahmi I, Ramzy AM and Amin Y (1996) Intracytoplasmic sperm injection using microsurgically retrieved epididymal and testicular sperm. Fertil Steril 65,566–572.

Meldrum DR, Chetkowski R, Steingold KA, de Ziegler D, Cedars MI and Hamilton M (1987) Evolution of a highly successful in vitro fertilization-embryo transfer program. Fertil Steril 48,86–93.

Menezo Y, Anker D and Salat-Baroux J (1985) Conception and realization of artificial dyed embryos for training in in vitro fertilization and embryo transfer (IVF and ET). Acta Eur Fertil 16,55–58.

Poindexter AN, 3rd, Thompson DJ, Gibbons WE, Findley WE, Dodson MG and Young RL (1986) Residual embryos in failed embryo transfer. Fertil Steril 46,262–267.

Schulman JD (1986) Delayed expulsion of transfer fluid after IVF/ET. Lancet 1,44.

Woolcott R and Stanger J (1997) Potentially important variables identified by transvaginal ultrasound-guided embryo transfer. Hum Reprod 12, 963–966.

Woolcott R and Stanger J (1998) Ultrasound tracking of the movement of embryo-associated air bubbles on standing after transfer. Hum Reprod 13,2107–2109.

Submitted on November 13, 2003; accepted on September 29, 2004